COMMONWEALTH OF AUSTRALIA

IN THE MATTER OF : Australian Patent Application 696764 (73941/94). In the name of: Human Genome Sciences Inc.

-and-

IN THE MATTER OF: Opposition thereto by Ludwig Institute for Cancer Research, under Section 59 of the Patents Act.

STATUTORY DECLARATION

I, Tom Rapoport of Harvard Medical School, of Harvard University, Boston, Massachusetts, United States of America, declare as follows:

1. I am currently a Professor of Cell Biology for the Howard Hughes Medical Institute in the Department of Cell Biology at the Harvard Medical School in Boston, Massachusetts. In 1995, I was appointed Professor of Cell Biology at Harvard Medical School. Prior to that appointment, I was Group Leader at the Max-Delbruck-Center for Molecular Medicine, Germany, from 1992-1994. From 1985-1992, I was the Group Leader at the Institute for Molecular Biology, Germany. From 1972-1985, I was a Research Assistant at the Zentralinstitut for Molekularbiologie der Akademie der Wissenschaften der DDR.

2. Since the early 1970s, my research has focused on protein processing and intracellular transport and the role of signal sequences in protein translocation across cell membranes, as evidenced by my curriculum vitae, which lists the publications that I have authored or co-authored. My research in the area of protein translocation has encompassed bacterial, yeast and mammalian systems. I am also broadly interested in examples of nonclassical secretion

pathways of mammalian proteins, including, fibroblast growth factor (FGF). Now shown to me and marked "TP-1" is a copy of my curriculum vitae.

3. I have been asked by the Patent Attorneys representing Human Genome Sciences ("HGS") to review Australian Patent Application Au-B-696764 (73941/94) in the name of HGS, entitled "Vascular Endothelial Growth Factor-2" ("the HGS patent specification"), which claims priority and has a virtually identical specification to U.S. application no. 08/207,550, filed March 8, 1994. I have also been asked to provide my comments and opinions as to what the patent specification would provide or teach to one of ordinary skill in the art of protein processing as of the earliest filing date of the HGS patent specification, March, 1994. For purposes of this analysis, I considered not only what I knew and appreciated at the relevant time, but what was expected to be known by graduate students and postdoctoral fellows who were in my laboratory at the relevant time. I have also been asked to comment on the state of the art of signal sequences and protein processing as of the earliest filing date of the HGS patent specification, March, 1994.

4. By the late 1970s, secreted proteins and the signal sequences required to route these proteins through the cell were well understood. For example, it was known that specific signals, often called signal sequences, were required to direct secreted proteins outside of the cell. These signal sequences were known to be located at the N-terminus of the secreted protein and removed either during or shortly after translocation of the protein across the endoplasmic reticulum and to the outside of the cell.

5. By 1994, it was known that, in mammals, signal sequences were typically located at the beginning of the secreted protein, often comprising 20 amino acids, and were characterized by a stretch of at least six or seven consecutive hydrophobic amino acids, the majority of which are leucine residues. Outside of the consecutive hydrophobic residues, there were other known requirements

for signal sequences, including the type of residues which provide the information to direct the cell to remove the signal sequence, i.e., the cleavage site. This cleavage site was known to contain small aliphatic residues, such as alanines at positions -1 and -3 to the N-terminus of the cleavage site, and was known to be usually found five to seven residues downstream from the stretch of consecutive hydrophobic residues.

6. By 1994, researchers were able to characterize signal sequences by their hydrophobic nature. Such sequences were often identified by an inspection of the amino acid sequence. Alternatively, computer programs, such as P SORT and SIGNAL P were, and still are today, standard tools used to predict the presence or absence of signal sequences. There was however no certainty with such approaches.

7. I have reviewed and analyzed the polynucleotide, and amino acid sequence, identified by HGS to encode the human VEGF-2 protein, as set forth in Figure 1 of the HGS patent specification. The human VEGF-2 protein is described in the HGS patent specification as structurally related to the PDGF/VEGF family of growth factors, a known family of secreted proteins. The HGS patent specification further states that the VEGF-2 polynucleotide is predicted to contain an open reading frame of approximately 350 residues, which encodes VEGF-2. (See, the HGS patent specification at page 5, lines 25-27). The specification reports that at the amino acid level VEGF-2 exhibits the highest homology to vascular endothelial growth factor (30% identity), followed by PDGF alpha (23%) and PDGF beta (22%). (See, the HGS patent specification at page 5, lines 28-31). The VEGF-2 protein was further characterized in the HGS patent specification as containing eight cysteines which are conserved among all four members of the family, and in addition also contains the conserved or signature motif PXCVXXXRCXGCCN which is found in all members of the PDGF/VEGF family. (See, the HGS patent specification at page 5, lines 31-33). The HGS patent specification speculates that the first 24

residues of the 350 amino acid sequence may encode a signal sequence. (See, the HGS patent specification at page 5, lines 26-27).

8. Attached as "Annexure TP-2" is an annotated copy of Figure 1 of the HGS patent specification which sets forth the nucleotide and amino acid sequence of VEGF-2 identified by HGS. The figure is further annotated to indicate specific amino acid residues and those portions of the VEGF-2 sequence which will become relevant throughout this declaration. For consistency of nomenclature, the numbering of amino acid residues will be referred to in the context of what is now known as the 419 amino acid form of VEGF-2. As shown in Annexure TP-2, the 350 amino acid form of VEGF-2 corresponds to amino acid residues 70 to 419 of the 419 form of VEGF-2.

9. By 1994, had I or any one skilled in the art, such as any Ph.D. scientist or candidate in my laboratory, been presented with the HGS patent specification, (see ¶ 7 above) one would recognize that the VEGF-2 protein is a secreted protein. Based on the characterization of the VEGF-2 protein set forth in the HGS patent specification, one would recognize that the protein was a member of the PDGF/VEGF family of growth factors. The PDGF/VEGF family of growth factors, like other growth factors, must be secreted in order to exert their growth promoting or mitogenic effects. Since all previously identified members of the PDGF/VEGF family were known to be secreted, one would expect the newly identified VEGF-2 to also be secreted.

10. Had I or any skilled artisan been presented with the HGS patent specification in 1994 and wanted to express the VEGF-2 protein, one would proceed with inspecting the 350 amino acid sequence set forth in Figure 1 to identify a potential signal sequence. As I have already noted, by 1994, this was routinely achieved by visual inspection or could be achieved with the aid of a computer program. Upon inspection of the N-terminal portion of the 350 amino acid sequence, I did not observe the typical conserved motif of a signal sequence.

In fact, the N-terminal portion of the 350 amino acid sequence is not very hydrophobic and contains many charged residues. However, provided with the strong evidence that the 350 amino acid sequence was a secreted protein based on the teaching and recognition of the HGS patent specification that it is a member of a family of secreted growth factors (see ¶ 7 above), I would not rule out that the 350 amino acid sequence may contain an atypical signal sequence. Atypical signal sequences are not without precedent. For example, as of March 1994, it was known that ovalbumin, a secreted protein, contains an atypical and uncleaved signal sequence that is not immediately obvious on simple inspection.

11. The possibility of additional upstream coding regions from the sequence disclosed in Figure 1 of the HGS patent specification would not have dissuaded me nor do I believe it would have dissuaded my Ph.D. students or post-doctorate students from attempting to express the 350 amino acid sequence. By 1994, it was well established that the PDGF/VEGF family of growth factors were expressed initially as precursor proteins which underwent proteolytic processing resulting in a mature form of the protein. Thus, I would predict VEGF-2 to be expressed in a similar way. The 350 amino acid sequence set forth in Figure 1 contains those conserved motifs which are signature motifs for an active form of the protein belonging to the PDGF/VEGF family. Thus in 1994, I would have predicted the protein encoded by the sequence disclosed in Figure 1, containing motifs characteristic of the PDGF/VEGF family, to be secreted and biologically active.

12. By 1994, in order to ensure secretion of the VEGF-2 sequence disclosed in Figure 1, I would have engineered a heterologous signal sequence upstream from the methionine at position 70. Indeed, this approach is specifically taught in the HGS patent specification (at page 14, lines 6-23). Moreover, it would have involved routine practice in 1994 to select a strong signal sequence, such as that of human growth factor or insulin growth factor, and to

engineer such a construct to achieve expression and secretion of the gene product. Even given the possibility that the 350 amino acid sequence may contain an atypical signal sequence, I would still have utilized a strong signal sequence to ensure efficient secretion of the protein, and it would have been standard practice to do so. The upstream signal sequence would be expected to override any weaker signal sequence that may be present downstream, and none of the segments of amino acids in the 350 amino acid sequence are hydrophobic enough to prevent the secretion of the protein through the endoplasmic reticulum and to the outside of the cell. Thus, I would have fully expected to achieve expression and secretion of the VEGF-2 protein using a heterologous signal sequence.

13. The expectation that engineering a signal sequence upstream of the sequence set forth in Figure 1 would result in the expression and secretion of a biologically active protein as set forth in the HGS patent specification, has in fact been subsequently confirmed by one skilled in the field of VEGF-2, Dr. Kari Alitalo. Drs. Kari Alitalo and Vladimir Joukov are named as co-inventors of U.S. Patent No. 6,130,071; issued October 10, 2000, entitled: "Vascular Endothelial Growth Factor C (VEGF-C) Cys 156 Protein and Uses Thereof" (the "Alitalo Patent"). It is my understanding that VEGF-2 and VEGF-C are terms used to refer to the same molecule. The Alitalo Patent describes several fragments of VEGF-C which were engineered to be expressed and secreted from cells. Cells were engineered to express the VEGF-2 fragments by fusing a signal sequence to the nucleotide sequence encoding the protein fragment. Secreted protein fragments were obtained from the cell culture medium and tested for activity. The working examples of the Alitalo Patent demonstrate that VEGF-C fragments spanning residues 103-419 or 112-419 (see Annexure TP-2) which are fused in frame to a signal sequence are secreted into the culture medium. (Alitalo Patent, column 47, lines 44 to 48). The Alitalo Patent also demonstrates the use of a heterologous signal sequence to ensure secretion of a fragment of VEGF-C. A fragment

spanning residues 104-213 (see Annexure TP-2) was fused in frame to a heterologous signal sequence which resulted in the secretion of a biologically active form of VEGF-C. (Alitalo Patent, column 46, lines 5 to 10). Furthermore, a publication subsequent to the priority date of the HGS patent specification, which Dr. Alitalo co-authored, Joukov et al, 1997, EMBO J. 16:3898-3911 ("Joukov"), describes two VEGF-C mutants in which the native VEGF-C signal sequence was fused in frame with residues 103-419 or residues 103-227. Both VEGF-C mutants were shown to be secreted into the culture medium, (Joukov at page 3901) using experimental procedures that were routine by 1994. Thus, both Alitalo and Joukov confirm that the fusion of a signal sequence in frame to VEGF-2 fragments will result in secreted protein fragments.

14. The expectation that I would have had from reading the HGS patent specification and the expectation that I would have expected others to have had is that the sequence set forth in Figure 1 does indeed contain the conserved motifs which would confer biological activity to the VEGF-2 protein as set forth in the HGS patent specification (see ¶ 7 above) has also been subsequently confirmed by Dr. Alitalo. In the Alitalo Patent and Joukov, the VEGF-2 protein fragments were tested for VEGF-C biological activity in different assays. The Alitalo Patent expressed and assayed several fragments in this manner, including fragments spanning residues 103-225, 103-419, 104-213, and 112-419 (see Annexure TP-2), and as described in ¶ 13, above. Each of the fragments assayed were found to be biologically active. Based on these observations and sequence comparisons of the VEGF family, the Alitalo Patent states that still smaller fragments of the 419 amino acid sequence of VEGF-C will retain biological activity. (Alitalo Patent, column 47, line 57 to column 48, line 2). The Alitalo Patent further states that a protein which retains the conserved motif RCXXCC (e.g., a polypeptide comprising from about residue 161 to about residue 211, see Annexure TP-2) is postulated to

retain biological activity. (Alitalo Patent, column 47, line 57 to column 48, line 2).

15. As observed in the Alitalo Patent and Joukov, VEGF-C fragments spanning residues 103-225, 103-419, 104-213, and 112-419 (see Annexure TP-2) fused in frame with signal sequences contained sufficient information to be processed by the cell to result in a biologically active form of VEGF-C. Furthermore, Alitalo also predicts that a VEGF-C fragment spanning residues 161-211 of VEGF-C (see Annexure TP-2) contains sufficient information to confer biological activity. Thus, if these VEGF-C fragments spanning residues 103-225, 103-419, 104-213, 112-419 and 161-211 contain sufficient information to allow for processing of a biologically active form of VEGF-C, the longer sequence set forth in the HGS patent specification which spans residues 70-419 (see Annexure TP-2), fused in frame to a signal sequence, should also contain sufficient information to be processed by the cell to result in a biologically active form of VEGF-C. According to the Alitalo Patent and Joukov, proteolytic processing of VEGF-C appears to differ depending on the cell used to achieve expression of the VEGF-C protein (Joukov at ¶ spanning pages 3906-3907). However, both Alitalo and Joukov demonstrate that regardless what type of mammalian or yeast cells are used to express VEGF-C, a biologically active VEGF-C protein is obtained. (Joukov at ¶ spanning pages 3906-3907; Alitalo Patent at column 46, lines 5-10). Consequently, regardless of the cell line used to express VEGF-C and the exact proteolytic processing or glycosylation that results, a biologically active fragment of VEGF-C can still be obtained. Thus, consistent with the teaching of the HGS patent specification, residues 70-419 as set forth in Figure 1 also contain sufficient information to confer VEGF-C biological activity.

16. The Alitalo Patent and Joukov have clearly confirmed that the teaching of the HGS patent specification, that the fusion of a signal sequence to a fragment of VEGF-C will result in the successful secretion of that fragment from the cell

into the culture medium. Thus, according to the teaching of the HGS patent specification, residues 70-419 as set forth in Figure 1 fused in frame to a signal sequence will result in its successful secretion from the cell into the culture medium.

17. In sum, as described in the HGS patent specification, Dr. Alitalo has subsequently demonstrated and/or predicted that VEGF-C fragments spanning residues 103-227, 103-419, 104-213, 112-419 and 161-211 (see Annexure TP-2) when fused in frame to a signal sequence result in a secreted gene product which retains VEGF-C biological activity. Thus, the sequence set forth in the HGS patent specification, spanning residues 70 to 419 of VEGF-C (see Annexure TP-2) fused in frame to a signal sequence should also result in a protein which is secreted and retains VEGF-C biological activity.

18. In my opinion, I or one skilled in the art would identify the VEGF-2 protein as a novel member of the PDGF/VEGF family of growth factors, and as such, would recognize that VEGF-2 is also a secreted growth factor, based on the HGS patent specification in combination with the state of the art as of March, 1994. I or one skilled in the art would recognize the utility in using a heterologous signal sequence to achieve expression and secretion of the VEGF-2 protein, based on the HGS patent specification in combination with the state of the art as of March, 1994. Hence, I or one skilled in the art following the teaching of the HGS patent specification coupled with the knowledge of the art at March 1994, would have predicted and expected to achieve expression and secretion of a protein which retains VEGF-2 biological activity and it would have been obvious to carry out those experiments to achieve that purpose.

AND I declare further that all statements made in this Declaration of my own are true in every particular, and that all statements made on information and belief are believed to be true.

Sworn by the said Tom Rapoport, _____,

at Boston Massachusetts, on this 13th day of ~~November~~ December 2000;

before me: _____.
Notary Public

LORNA L. FARGO
MY COMM. EXPIRES 12-01-06

Curriculum vitae

Name: Tom A. Rapoport

Address: Howard Hughes Medical Institute
Harvard Medical School
Department of Cell Biology
240 Longwood Avenue
Boston, MA 02115-6091

private address:

65 Greenough Street
Brookline, MA 02445

Phone: (617) 432-0637 (office)
(617) 566-6575 (home)

Personal data: born 06/17/47 in Cincinnati (USA)
married, 3 children

Education:

1965 - 1966	High school specialized in mathematics and natural sciences at the Humboldt-University Berlin, graduation with "honors"
1966 - 1972	Study of chemistry and biochemistry at the Humboldt-University Berlin, graduation with "honors"
1972	Ph.D. for work on "The mechanism of the inorganic pyrophosphatase of baker's yeast"
1977	"Habilitation" for work on "The development of a control theory for the mathematical modelling of metabolic pathways"

Areas of research:

1969 - 1972	enzyme kinetics, enzymology, protein purifications

	1970	- 1980	mathematical modelling of metabolic systems, studies on the regulation of the glycolysis in erythrocytes
	1972	- 1980	biosynthesis of carp insulin, cloning of the cDNA for carp insulin, expression of the gene in E.coli, oocyte injection of mRNAs
	1972	-	intracellular protein transport, transport of proteins across the endoplasmic reticulum membrane, membrane biogenesis
Academic positions:	1972	- 1985	Research Assistant at the Zentralinstitut für Molekularbiologie der Akademie der Wissenschaften der DDR
	1985	- 1992	Professor of Cell Biology
	1985	- 1992	Group Leader at the Institute for Molecular Biology
	1992	- 1994	Group Leader at the Max-Delbrück-Center for Molecular Medicine
	1995	- 1997	Professor of Cell Biology at Harvard Medical School
	1997	- present	HHMI Professor of Cell Biology at Harvard Medical School

Memberships: German Biochemical Society
Academy of Sciences
Academea European
EMBO
American Society for Cell Biology

Honors: Johannes-Müller-prize of the Society for Experimental Medicine

Rudolf-Virchow-prize

Sabbatical:	1982	3 month stay in the laboratory of Dr. G. Blobel (Rockefeller University New York)

Major Committee Assignments:

1996-1997	Standing Committee on Promotions, Reappointments and Appointments in the Faculty of Medicine at Harvard Medical School
1996-present	Various promotion committees
1996-present	Various Ph.D. Thesis Committees
1997-1999	Vice Chair/Chair, Gordon Research Conference on Molecular Membrane Biology
1999-present	NIH study section (permanent member)

Editorial Boards:

1980-1988	FEBS Letters
1989-present	EMBO Journal
1989-present	The Journal of Cell Biology
2000	EMBO Reports

Teaching Report:

1996-1998	Co-director, Cell Biology 201 course
1999-2000	Director, Cell Biology 201 course
1998-1999	CBC course (medical students)
1998-1999	Molecular Machines course

List of publications

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The regulatory principles of the glycolysis of erythrocytes in vivo and in vitro

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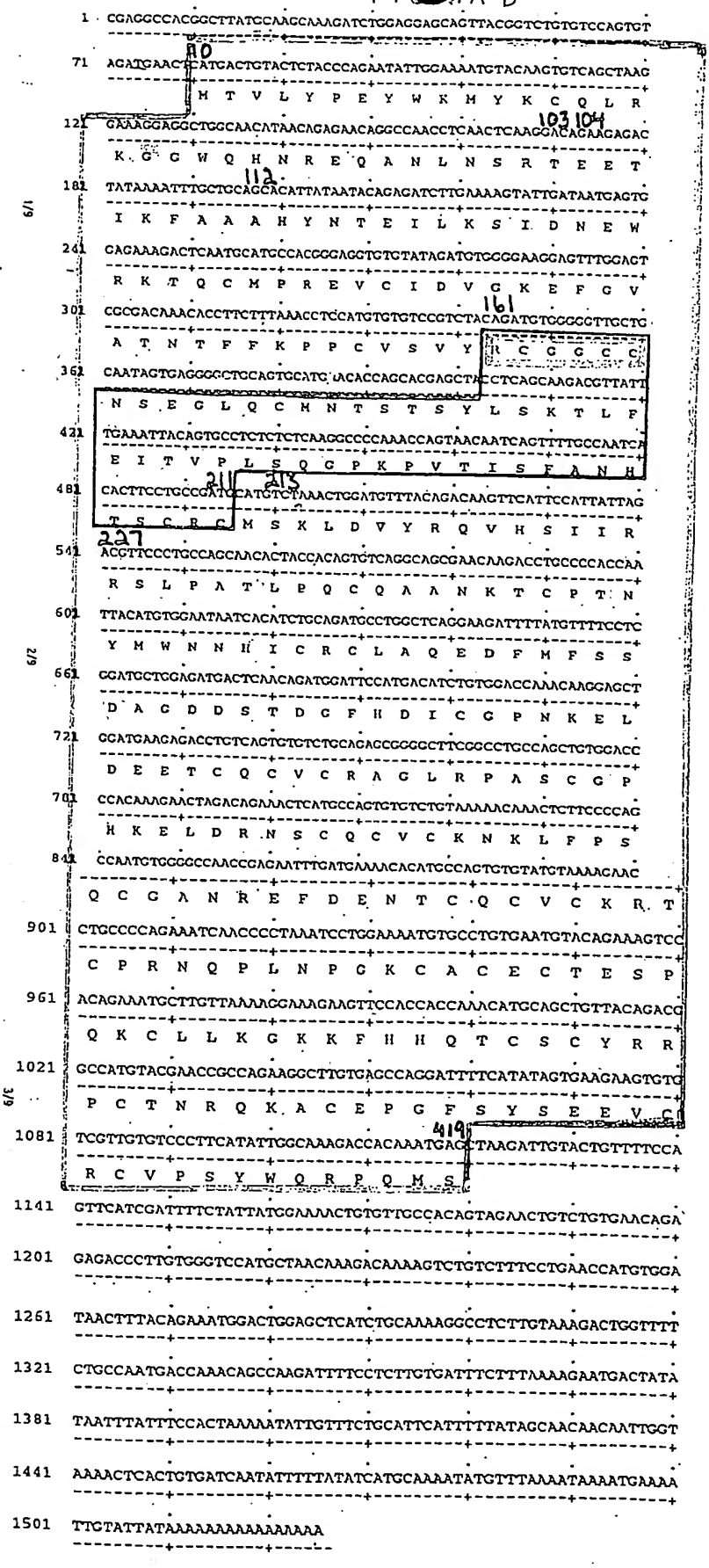
FIGURE 1A-D



HGS VEGF-2 350 amino acid sequence (70-419)

Minimum sequence required to maintain VEGF-C biological activity as identified by Alitalo

RCXXCC conserved motif

Alitalo VEGF-C fragments:
- 103-227
- 103-419
- 104-213
- 112-419

ANNEXURE TP-2